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UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
NATIONAL VETERINARY SERVICES LABORATORIES
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SAM 120

9 CFR 113.8	November 1, 1991	Bovine Respiratory Virus *in vitro* Potency Assay
Standard Requirement	New	Agent

SUPPLEMENTAL ASSAY METHOD

FOR

THE *IN VITRO* POTENCY ASSAY OF

BOVINE RESPIRATORY VIRUSES IN KILLED VACCINES

A. SUMMARY

This is an *in vitro* assay for determining the potency of killed vaccines containing Bovine Rhinotracheitis (BRV), Bovine Virus Diarrhea (BVD), Bovine Parainfluenza 3 (PI_3) and Bovine Respiratory Syncytial Viruses (BRSV) relative to a reference vaccine.

B. MATERIALS

1. Ninety-six (96) well microtiter plate[a].

2. Coating Buffer: 0.05 M sodium carbonate/bicarbonate pH 9.6. Store at 4° C; use within 5 days.

   a. 1.59 g Na_2CO_3

   b. 2.93 g $NaHCO_3$

   c. Distilled H_2O, qs to 1 L

   d. Adjust pH to 9.6 ± 0.1

3. Anti-viral agent capture antibody, using one capture antibody only, and assay one viral fraction in each test. Reference quantities are available from National Veterinary Services Laboratories (NVSL). Dilute in coating buffer according to instructions.

   a. BRV antibody

   b. BVD antibody

   c. Bovine PI_3 antibody

   d. BRSV antibody

4. Blocking Buffer: 1% casein in coating buffer. Store at 4° C; use within 5 days.

   a. 1 g casein[b]

   b. 100 ml coating buffer

   c. Heat 2-3 min until boiling in a microwave on high setting.

5. 0.01 M PBS. Store at 4° C.

   a. 1.33 g Na_2HPO_4

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[a] Immulon-2, Dynatech Laboratories, Inc. No endorsement expressed or implied.

[b] Sigma Chemical Co. No endorsement expressed or implied.

b. 0.22 g $Na_2H_2PO_4 \cdot H_2O$

c. 8.5 g NaCl

d. Distilled H_2O, qs to 1 L

e. pH 7.2

f. Autoclave 15 min at 121° C.

6. Diluent Buffer: 1% casein in 0.01 M PBS. Prepare fresh each time test is performed.

a. 1 g casein

b. 100 ml 0.01 M PBS

c. Heat 2-3 min until boiling in a microwave on high setting.

7. Wash Reagent: 0.05% Tween-20[c] in 0.01 M PBS

8. Secondary antibody: Available from NVSL unless noted. Dilute in dilutent buffer according to instructions.

a. BRV monoclonal antibody (Mab):

(1). 1B8

(2). 2H6

b. BVD biotin-labeled polyclonal antibody

c. PI_3 Mab[d]:

(1). 240-12D

(2). 260-10B

d. BRSV Mab 8G12

9. Conjugate:

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[c] Bio-Rad Laboratories. No endorsement expressed or implied.

[d] Chemicon International, Inc. Temecula, CA, Cat. #MAB855-3. No endorsement expressed or implied.

a. Horseradish peroxidase-labeled goat anti-mouse IgG[e], diluted 1:3000 in 0.01 M PBS, is to be used with the BRV, PI_3 and BRSV Mabs.

b. Peroxidase conjugated streptavidin[f], diluted 1:4000 in 0.01 M PBS, is to be used with biotin-labeled polyclonal BVD antibody.

10. Substrate: 2,2'-azino-di(3-ethylbenzthiazoline sulfonic acid) (ABTS)[g]

a. Mix equal parts of Solution A and Solution B as supplied just before use in the *in vitro* test.

11. Reference vaccine(s) to be supplied by the licensee. The reference vaccine(s) should have the same dose size and composition as the test serial, be validated in a host immunogenicity study and shown to work in a parallel line assay. (Refer to the Guidelines for measuring the relative potency of veterinary biologics by ELISA, dated April 26, 1991.)

C. METHOD

1. If the test serial vaccine is treated to release antigen from adjuvant, it shall be a method acceptable to Animal Plant Health Inspection Service and shall be applied to the reference vaccine as well.

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[e] Fisher Scientific, Cat. #OB1120 HRPO. No endorsement expressed or implied.

[f] Zymed Laboratories, Inc. Cat. #43-4323. No endorsement expressed or implied.

[g] Kirkegaard & Perry Laboratories Inc. Cat. #506200. No endorsement expressed or implied.

2. Coat microtiter plate with 100 µl/well of capture antibody diluted in coating buffer. Cover with sealing tape. Incubate overnight at 4° C.

3. Decant capture antibody. Blot inverted plate on paper towels. Add 100 µl/well of blocking buffer. Incubate 1 hr at room temperature (RT).

4. Rinse microtiter plate 3 times using 200 µl/ well of wash reagent. Remove residual wash reagent by blotting inverted plate on paper towels after the 3rd wash. Prevent drying of plate between reagent additions.

5. Prepare serial 2-fold dilutions of the reference and test serial vaccines in a separate dilution plate.

   a. Add 150 µl/well of diluent buffer to rows B-H of a 96-well plate.

   b. Add 300 µl/well of reference vaccine to A1 through A3 of the dilution plate. Add 300 µl/well of the test serial vaccine to A4-A6 of the dilution plate. Additional test serials may be tested in A7-A9 and A10-A12.

   c. Using a multichannel pipet transfer 150 µl of row A to row B. Thoroughly mix contents of wells in row B. Transfer 150 µl to the corresponding wells in row C. Note: One set of pipet tips may be used for diluting the reference and serial vaccines.

   d. Thoroughly mix contents of wells in row C. Transfer 150 µl to the corresponding wells in row D.

   e. Continue serial 2-fold dilutions through row G. After thoroughly mixing the contents in wells G, discard 150 µl

from row G. The dilutions of the vaccines range from undiluted to 1:64. Note: Other dilution schemes for the reference vaccine and test serial may be used to obtain optimal optical density (OD) readings.

f. Row H is the blanking row.

6. Use the multichannel pipet to transfer 100 µl aliquots from row H of the dilution plate to the corresponding row H of the capture antibody coated plate. Continue transfer for all rows of the dilution plate from row G through row A. One set of pipet tips may be used to transfer. Incubate 1 hr at 37° C on a microtiter plate shaker[h].

7. Rinse plate again as in step 4.

8. Add 100µl/well of secondary antibody diluted in diluent buffer to all wells of the plate. Incubate 1 hr at 37° C.

9. Rinse plate again as in step 4.

10. Add 100 µl/well of conjugate diluted in 0.01 M PBS. Incubate 30 min at 37° C.

11. Rinse plate 3 times with 0.01 M PBS without tween-20. Remove residual PBS by blotting inverted plate on paper towels after the 3rd wash.

12. Add 100 µl/well of ABTS. Incubate 45 min at RT.

13. Read plate on an ELISA reader with 405 nm for the test filter and 490 nm for the reference filter.

14. Blank reader on well(s) containing all reagents except the fraction being tested (row H). If reader blanking is not none, the mean OD value obtained from the blank(s) should be subtracted

[h] Lab-Line Instruments Inc. No endorsement expressed or implied.

from all other OD values before any data analysis.

15. Interpretation: A test that results in no valid lines is considered a no test and may be repeated. The reported relative potency (RP) of the test serial is the highest RP from the top 3 scores calculated using Standard Assay Method 318[i]. Test serials with RP ≥ 1.0 are considered satisfactory. Retest procedures are covered under the Code of Federal Regulations title 9, 113.8. Test serials with a RP < 1.0 may be retested up to a total of 3 times. At least 50% of all valid tests must be satisfactory for the test serial to be considered satisfactory. If the test serial is not retested the serial is considered unsatisfactory.

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[i] Relpot; USDA, APHIS, National Veterinary Services Laboratories. Current version.